Hedgehog signaling pathway: the must, the maybe and the unknown

Paul Zarogoulidis[1], Katerina Zarampouka[1], Haidong Huang[2], Kaid Darwiche[3], Yong Huang[4], Antonis Sakkas[5], Konstantinos Zarogoulidis[1]

[1]Pulmonary Department-Oncology Unit, "G. Papanikolaou" General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Department of Respiratory Diseases, Changhai Hospital; First Affiliated Hospital of the Second Military Medical University, Shanghai, China; [3]University Pulmonary Department-Interventional Unit, Rurhland Clinic, University of Duisburg-Essen, Essen, Germany; [4]Respiratory Department, Chongqing Zhongshan Hospital, Chongqing 400013, China; [5]Pathology Department, "G. Papanikolaou" general Hospital, Thessaloniki, Greece

ABSTRACT Lung cancer treatment consists from the basic chemotherapeutic drugs (e.g., platinum analogues) and from pharmaceuticals targeting the different genome of lung tumors (e.g., tyrosine kinase inhibitors). During the last years pharmaceuticals targeting the tumor mutations are approved for first line treatment since they have provided increased overall survival in comparison to standard chemotherapy treatment. Furthermore, due to the increased interest in unrevealing the mechanisms of cell mutation, tumor evolution and tumor cell maintenance the hedgehog pathway has been elicited. Along with Notch and Wnt these three pathways are responsible for progenitor cell development and pulmonary organogenesis. Inhibitors of this pathway have been discovered and their application in the clinical practice is being investigated. However, further understanding of the mechanisms of regulation is needed.

KEY WORDS Hedgehog; molecular pathways; lung cancer

J Thorac Dis 2013;5(2):195-197. doi: 10.3978/j.issn.2072-1439.2013.03.02

The last decade should be addressed as the era of the genome. Several therapies targeting specific mutations to lung cancer have been approved as first line treatment (1,2). However, tumor is the product at the last stage of a process that started several steps before. Therefore an increasing effort is being made to identify all the implicated pathways and mechanisms. This effort has elicited the hedgehog (Hh) pathway which was first observed in *Drosophila* larva (3). The Hh is responsible for regulating the embryonic stem cells in the early life development of the respiratory system. Further factors included in the normal lung branching of the respiratory system are: (I) bone morphogenic protein (BMP), (II) fibroblast growth factor (FGF) and Ras modifiers. These three additional mechanisms have been well identified in a *Drosophila* model (4). The first observation between cancer and Hh was observed with studies of Gorlin's Syndrome. The activation of Hh is observed in many cancer types; skin, brain, gastrointestinal, breast, prostate (5) and recently small cell lung cancer (SCLC) (6,7) and non-small cell lung cancer NSCLC) (8-11). In the study by Raz *et al.* the Hh signaling pathway was evaluated along with the aldehyde dehydrogenase family 1 member A1 (ALDH1A1) in stage I-II NSCLC (10).

The Hh pathway is a mechanism of repair/regeneration in adult respiratory tissue, and is deregulated with smoking (12). Since SCLC is observed in heavy smokers it is evident why Hh pathway deregulation induces mostly in this type of cancer tumorigenesis. The Hh signaling is responsible for the formation of the respiratory system (13-15), it has been observed that when cyclopamine and jervine where administered in pregnant animals the respiratory system formation presented abnormalities (16). Hh is responsible for the main structure of the respiratory system, regarding the respiratory epithelium which consists of basal cells, beating cilia and clara cells their regulation is performed through the Notch and Wnt signaling (17). These three mechanisms continue to work as a cluster to repair and regenerate the injured respiratory system, only in the epithelial progenitor cells. The persistent airway epithelial injury activates the Hg signaling and promotes the expansion of the airway epithelial progenitor cells (17). It has been previously observed in surgically resected patients with NSCLC that the Hh signaling pathway was activated and overexpressed in the tumor while in the normal lung tissue such observation was not made (8). The Hh signaling

Corresponding to: Paul Zarogoulidis, M.D, Ph.D. Pulmonary Department-Oncology Unit, "G. Papanikolaou" General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece. Email: pzarog@hotmail.com.

Submitted Feb 07, 2013. Accepted for publication Mar 07, 2013.
Available at www.jthoracdis.com

ISSN: 2072-1439

consists of three variants; desert (Dhh), Indian (Ihh) and sonic (Shh). The Shh is formed after a 45 kDa protein is converted to a 19 kDa (18). In addition, there are two receptors; the transmembrane receptor-patched homolog 1 and 2 (Ptch 1 and 2), and a G protein coupled receptor the smoothened (Smo). Finally it consists of a cytoplasmic complex which the glioma-associated oncogene homolog (Gli) family (19). The Shh and Gli 1 are expressed in lung cancer both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). However, it has been observed that both Shh and Gli are observed in 50% of SCLC and only in 10% of NSCLC (17). Gli 3 has been found to be expressed in NSCLC cancer cell lines (7), and in one study Gli 2 was found also expressed (8). Gli target molecules have been extensively analyzed for their connection with squamous cell carcinoma. Out of 223 genes the 37 were associated with squamous cell carcinoma. CDC45, CDK1, CSTA, HDAC1, JAG1, JUP, KRT15, KRT17 have been found up-regulated and CXCL2, FHL1, FOXA2, PAPSS2, RHOB, SAP18 and SYNE1 were down-regulated (9). All the dysfunctional genes are responsible for the deregulated production of enzymes, growth factor, cytokines, kinases, transcription regulators and transporters. These findings were found to differ among different cell lines (7) and the inhibition of Hh was observed only in SCLC and not NSCLC, because in SCLC both Shh and Gli are expressed (17). It has been observed that genetic instability of cancer cell lines may cause phenotypic changes (7). A possible explanation is that in NSCLC the Hh is regulated by other independent mechanisms (8). Hh activation was also observed higher in squamous cell carcinoma (8,9). The Hh interacting protein (HIP) inhibits the Hh pathway however it is down-regulated by neo-angiogenesis which occurs in malignant transformation and evolution. Malignant cell proliferation is maintained once the mechanism of Hh regulation has been diverted. Ectopic expression of Shh leads to ectopic Hh interacting protein (20). In the study by Olsen *et al.* (21) it was demonstrated that angiogenesis was associated with Hh deregulated by inhibition of HIP. Moreover, the Hh pathway is influenced by other relevant cancer pathways such as the PRKCD which activates the ERK signaling. In specific the ERK signaling up-regulates the Gli transcription (22). Recent studies have also identified that apart from the Wnt and Notch, the down- regulation of Hox genes A3 and B4 are Hh-dependent Stat 3 (23). Epidermal growth factor receptor mutation has been also associated with Hh overexpression (24). Forkhead Box a family of transcription factors responsible for cell proliferation (25) was investigated for association in the Hh pathway signaling (8). It was observed that FOXM1 which is involved in the carcinogenesis of many human tumors is associated with Gli 1, Gli 2, SMO, PTCH 1 and Shh in NSCLC (8,26). FOXM1 maybe one of the factors affecting the Hh activity specifically in NSCLC. However, correlations in tissue have to be made along with other mechanisms stated in the current published literature.

Furthermore, interaction of the 7th membrane spanning receptor (or smoothened SMO) was observed in association with Ras related protein (Rab)-23, platelet derived growth factor receptor-α (PDGFR-α), hepatocyte nuclear factor 3-beta (HNF3β) (27). The SMO is localized in the cytoplasm and cancer cell membranes, however it is not able on its own to activate abnormal signal activation. Rab-23 is involved in the function of Gli in the nuclei, but not in the cytoplasm (27). Gli 1 which is found overexpressed in SCLC activates PDGFR-α, this observation demonstrates that this receptor is under the control of Hh signaling (27). The HNF3β may act as an independent factor for elevating Shh expression (27).

Stem cells have the ability to continuously regenerate and provide differentiated progenies. However, it is well known that a deregulation of this process with the additional influence of activated signaling pathways may induce malignant transformation (6). Microenvironment control is therefore essential. The current published literature indicates that the Hh signaling pathway is activated by continuous injury of the respiratory epithelium which is more common in heavy smokers. Therefore several groups have demonstrated an association between Hh deregulation of the Hh cascade in SCLC and squamous cell carcinoma which are both strongly associated with smoking. Further investigation towards the molecules produced from the Hh deregulation such; cytokines, enzymes and growth factors and their interaction with the Hh pathway are warranted. Finally, monoclonal antibodies are currently being developed. Cyclopamine and jervine are promising inhibitors of Hh pathway (17,28). Other molecules under evaluation are KAAD-cyclop, SANT1-4, CU61414, GANT 61, Tyrosine kinase inhibitors, p70S6K2casein kinases (CK1a and CK1e) glycogen synthetase kinase 3β (GSK3β) and protein kinase A (PKA) (e.g., foskolin), however resistance to this molecules has been observed due to differentiations in the Hh pathway due to mutation and cancer evolution (9,11,24,29-35). Targeting the cancer stem cells we may have the advantage of avoiding chemotherapy toxicity and impede a fundamental way to cancer evolution and maintenance.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Lyseng-Williamson KA. Erlotinib : a guide to its use in first-line treatment of non-small-cell lung cancer with epidermal growth factor-activating mutations. Mol Diagn Ther 2013;17:57-62.
2. Tateishi K, Ichiyama T, Hirai K, et al. Clinical outcomes in elderly patients administered gefitinib as first-line treatment in epidermal growth factor

receptor-mutated non-small-cell lung cancer: retrospective analysis in a Nagano Lung Cancer Research Group study. Med Oncol 2013;30:450.
3. Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in Drosophila. Nature 1980;287:795-801.
4. Hogan BL. Morphogenesis. Cell 1999;96:225-33.
5. Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. Nat Rev Drug Discov 2006;5:1026-33.
6. Watkins DN, Berman DM, Baylin SB. Hedgehog signaling: progenitor phenotype in small-cell lung cancer. Cell Cycle 2003;2:196-8.
7. Vestergaard J, Pedersen MW, Pedersen N, et al. Hedgehog signaling in small-cell lung cancer: frequent in vivo but a rare event in vitro. Lung Cancer 2006;52:281-90.
8. Gialmanidis IP, Bravou V, Amanetopoulou SG, et al. Overexpression of hedgehog pathway molecules and FOXM1 in non-small cell lung carcinomas. Lung Cancer 2009;66:64-74.
9. Shi I, Hashemi Sadraei N, Duan ZH, et al. Aberrant signaling pathways in squamous cell lung carcinoma. Cancer Inform 2011;10:273-85.
10. Raz G, Allen KE, Kingsley C, et al. Hedgehog signaling pathway molecules and ALDH1A1 expression in early-stage non-small cell lung cancer. Lung Cancer 2012;76:191-6.
11. Mizuarai S, Kawagishi A, Kotani H. Inhibition of p70S6K2 down-regulates Hedgehog/GLI pathway in non-small cell lung cancer cell lines. Mol Cancer 2009;8:44.
12. Lemjabbar-Alaoui H, Dasari V, Sidhu SS, et al. Wnt and Hedgehog are critical mediators of cigarette smoke-induced lung cancer. PLoS One 2006;1:e93.
13. Mahlapuu M, Enerbäck S, Carlsson P. Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signaling, causes lung and foregut malformations. Development 2001;128:2397-406.
14. Bellusci S, Furuta Y, Rush MG, et al. Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. Development 1997;124:53-63.
15. Litingtung Y, Lei L, Westphal H, et al. Sonic hedgehog is essential to foregut development. Nat Genet 1998;20:58-61.
16. Cooper MK, Porter JA, Young KE, et al. Teratogen-mediated inhibition of target tissue response to Shh signaling. Science 1998;280:1603-7.
17. Watkins DN, Berman DM, Burkholder SG, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 2003;422:313-7.
18. Ma Y, Erkner A, Gong R, et al. Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. Cell 2002;111:63-75.
19. Velcheti V, Govindan R. Hedgehog signaling pathway and lung cancer. J Thorac Oncol 2007;2:7-10.
20. Chuang PT, McMahon AP. Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. Nature 1999;397:617-21.
21. Olsen CL, Hsu PP, Glienke J, et al. Hedgehog-interacting protein is highly expressed in endothelial cells but down-regulated during angiogenesis and in several human tumors. BMC Cancer 2004;4:43.
22. Riobo NA, Haines GM, Emerson CP Jr. Protein kinase C-delta and mitogen-activated protein/extracellular signal-regulated kinase-1 control GLI activation in hedgehog signaling. Cancer Res 2006;66:839-45.
23. Sengupta A, Banerjee D, Chandra S, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. Leukemia 2007;21:949-55.
24. Mimeault M, Batra SK. Frequent deregulations in the hedgehog signaling network and cross-talks with the epidermal growth factor receptor pathway involved in cancer progression and targeted therapies. Pharmacol Rev 2010;62:497-524.
25. Laoukili J, Stahl M, Medema RH. FoxM1: at the crossroads of ageing and cancer. Biochim Biophys Acta 2007;1775:92-102.
26. Kim IM, Ackerson T, Ramakrishna S, et al. The Forkhead Box m1 transcription factor stimulates the proliferation of tumor cells during development of lung cancer. Cancer Res 2006;66:2153-61.
27. Huang S, Yang L, An Y, et al. Expression of hedgehog signaling molecules in lung cancer. Acta Histochem 2011;113:564-9.
28. Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425:851-6.
29. Chen JK, Taipale J, Young KE, et al. Small molecule modulation of Smoothened activity. Proc Natl Acad Sci U S A 2002;99:14071-6.
30. Sanchez P, Hernández AM, Stecca B, et al. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. Proc Natl Acad Sci U S A 2004;101:12561-6.
31. Price MA, Kalderon D. Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. Cell 2002;108:823-35.
32. Jia J, Amanai K, Wang G, et al. Shaggy/GSK3 antagonizes Hedgehog signalling by regulating Cubitus interruptus. Nature 2002;416:548-52.
33. Jia H, Liu Y, Xia R, et al. Casein kinase 2 promotes Hedgehog signaling by regulating both smoothened and Cubitus interruptus. J Biol Chem 2010;285:37218-26.
34. Raffel C, Jenkins RB, Frederick L, et al. Sporadic medulloblastomas contain PTCH mutations. Cancer Res 1997;57:842-5.
35. Tostar U, Malm CJ, Meis-Kindblom JM, et al. Deregulation of the hedgehog signalling pathway: a possible role for the PTCH and SUFU genes in human rhabdomyoma and rhabdomyosarcoma development. J Pathol 2006;208:17-25.

Cite this article as: Zarogoulidis P, Zarampouka K, Huang H, Darwiche K, Huang Y, Sakkas A, Zarogoulidis K. Hedgehog signaling pathway: the must, the maybe and the unknown. J Thorac Dis 2013;5(2):195-197. doi: 10.3978/j.issn.2072-1439.2013.03.02